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APPLICANT:	Ming-Tang Chang, et al.		
SERIAL NUMBER:	09/477,371	GROUP:	1638
FILING DATE:	January 6, 2000	EXAMINER:	C. Collins
TITLE:	**ANIMAL FEED WITH LOW PHYTIC ACID, OIL BURDENED AND PROTEIN LADEN GRAIN**		

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Date: April 22, 2003

Sir:

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

In support of the above-identified application, Jerry C. Weigel and Ming-Tang Chang state the following.

1. Jerry C. Weigel obtained his Bachelor of Science in Animal Science at the University of Nebraska in 1971 and did further studies in the Department of Animal Sciences of the University of Florida. He has worked in various positions in the animal nutrition industry for more than 30 years, and is currently the Manager of Nutrition for BASF Plant Science LLC. He has extensive experience in feed analytical methods and animal feed research. He is a co-inventor of the present application.

2. Ming-Tang Chang obtained his Bachelor of Science in Botany from the National Chung Hsing University, Taiwan, in 1968, his Master of Science in Food Crops from the National Chung Hsing University in 1973, and his Doctor of Philosophy in Agronomy from the University of Missouri in 1983, where he studied with M.G. Neuffer. He is the author or co-author of the publications listed below.

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Ming-Tang Chang is also a co-inventor of four U.S. patents and numerous pending applications worldwide. He has performed extensive research in the

area of seed mutagenesis, particularly mutagenesis using ethylmethanesulfonate (EMS), for more than 23 years. He is a co-inventor of the present application.

3. On the basis of his extensive experience, Ming-Tang Chang states that use of the EMS mutagenesis technique, first published in Neuffer and Coe, *Maize for Biological Research*, ed. By W.F. Sheridan, Plant Molecular Biology Association (1982), reliably and reproducibly produces point mutations in corn and other plant species. EMS-generated point mutations may be detected by appropriate screening assays, such as the known assays for detecting high oil, high protein, and low phytate mutant seeds of the present invention.

4. The methods of EMS mutagenesis and selection set forth in the instant application have reproducibly produced maize inbred lines having at least 5% by weight oil, at least 11% by weight protein, and at least a one third reduction in the phytic acid amount relative to wild-type maize seed. Using these methods, Applicants have selected 20 inbred lines, and within those 20 inbred lines, have produced more than 100 mutational events that were stable, low in phytate, and non-lethal. By selectively crossing among these lines, Applicants have obtained more than 50 candidate hybrid combinations which are low in phytic acid, high in oil, and high in protein. These candidate hybrids are actively undergoing testing for commercial suitability.

5. Using the methods set forth in the instant application, Applicants have made more than 50 non-lethal, mutant maize hybrids having at least 5% by weight oil, at least 11% by weight protein, and at least a one third reduction in the phytic acid amount relative to wild-type maize seed.

6. Analytical data representing the weight percent oil, weight percent protein, and percent phytate reduction for grain from a number of hybrids within the

scope of the present claims is set forth in Table 1 attached hereto. The hybrids described in Table 1 are presently in pre-commercial development by Applicants' assignee, BASF Plant Science LLC, as NUTRIDENSE-Low Phytate™ corn.

7. The methods of analysis for crude protein and total oil in NUTRIDENSE Low Phytate™ corn are conducted under the Guidelines of the American Organization of Analytical Chemists (AOAC). AOAC Official Method 988.05, Animal Feed, first implemented in 1988 and finally implemented in 1990, determines crude protein. This procedure was used for years in the analytical field prior to the official implementation of the method by AOAC. Oil is determined by AOAC Official Method 920.39, Fat (Crude) or Ether Extract in animal feeds, established in 1920.

8. Phytate content of the seed shown in Table 1 below was determined by a slightly modified version of the method described in the following publication: "*Latta, M and M Eskin. 1980. A simple and rapid colorimetric method for phytate determination. J. Agric. Food Chem. 28:1313-1315*". Kernel tissue extracts were prepared by crushing seed in a hydraulic press and extracting overnight in 1 mL of 0.65N HCl. Samples were vortexed before combining 20uls extract with 500uls Wade's reagent in a microcentrifuge tube. Samples were centrifuged (15 000 rpms, 2 mins) and 250 uls of supernatant was measured A490 on a microtiter plate using a BIO-TEK Instruments (Winooski, VT) model EL 340 automated microtiterplate reader. The phytate contents of both the low phytate mutant and its isoline counterpart were measured. The mutants are reported as a percent phytate content of the corresponding isoline. When available, phytate content measured on hybrid grain is reported (pedigrees 2,3,6 and 7). For the remaining hybrids, low phytate content of the grain was estimated on the basis of measured parent inbred content. Applicants have found that the phytate content of the hybrid grain can be predicted at plus or minus 5% of the measured value by taking

the average phytate content of the two parental inbreds that make up a given hybrid.

TABLE 1

Pedigree	Phytic Acid as a % of Isoline	Protein	Oil
BD68py0719x14UA013py0380	54.00%	12.30%	5.10%
BD68py0719xUO95py1656	22.00%	12.80%	5.10%
BD68py0719xUO95py1672	11.00%	12.90%	5.00%
BD68py0719xUO95py2148	16.00%	12.00%	4.60%
TR306py0510x14UA013py0380	63.00%	12.50%	5.20%
TR306py0510xUO95py2148	24.00%	13.20%	5.00%
TR306py0510xUO95py1672	24.00%	12.30%	5.40%
UO95py1656xTR306py0510	29.00%	12.30%	5.40%
TR329py0138x14UA013py0380	83.00%	12.90%	5.00%
TR329py0138xUO95py1656	53.00%	14.10%	5.10%
TR390py0478xUA013py0380	66.00%	12.00%	5.00%
TR390py0478xUO95py1656	35.00%	12.60%	5.40%
UO95py1656x14UA013py0380 +TR329py0138	68.00%	12.90%	5.00%
UO95py1656xTR390py0662	29.00%	12.20%	5.90%
UO95py1672xTR390py0478	26.00%	12.00%	5.80%
UO95py2148xTR390py0478	27.00%	12.00%	5.70%

* All values expressed on a 100% Dry Matter Basis`

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All statements made herein of declarants' knowledge are true, and all statements made on declarants' information and belief are believed to be true. The statements made herein were made with the knowledge that willful false statements and the like thereof so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Jerry C. Weigel

Dated: 22/4/03

Ming-Tang Chang

Dated: 22 April, 2003